

In The Claims

This listing of claims, in which deletions are ~~struck through~~ and additions underscored, will replace all prior versions, and listing, of the claims in the application.

Listing of Claims:

1-14. (Cancelled)

15. (Currently amended) A method for producing a protein of interest encoded by a gene under the control of an inducible promoter comprising the steps of:

(a) Generating a first mixture ~~Mixing a concentrated glucose solution~~ comprising between about 5% to about 75% glucose with and a cellulase preparation selected from the group consisting of a whole cellulase composition or beta-glucosidase enriched cellulase composition to give a first mixture, the beta-glucosidase activity in said first mixture being about 1.5 IU/ml to about 180 IU/ml;

(b) Incubating the first mixture at a temperature and for a sufficient time to produce an inducing feed composition comprising sophorose in a concentration ranging from 2 g/L to 25 g/L, and/or gentiobiose in a concentration ranging from 35 g/L to 60 g/L, and glucose; and

(c) Culturing a cell comprising a nucleotide sequence encoding a protein of interest under the control of a sophorose-inducible promoter or a gentiobiose-inducible promoter with said inducing feed composition, wherein said inducing feed has not been subjected to a purification step, in an amount effective to induce the production of said protein of interest ~~wherein said inducible promoter is a sophorose-inducible promoter or a gentiobiose-inducible promoter.~~

16. (Original) The method of claim 15 wherein the protein produced is an endogenous cellulase.

17. (Currently amended) The method of claim 15 wherein the cell has been has been genetically engineered to encode a protein of interest under the control of a sophorose-inducible

~~promoter or a gentiobiose-inducible promoter transformed with an expression construct comprising a promoter operably linked to a gene encoding a protein of interest.~~

18. (Cancelled)

19. (Original) The method of claim 17 wherein the promoter is a cellulase gene promoter.

20. (Original) The method of claim 19 wherein the promoter is the cbh1 promoter from *Trichoderma reesei*.

21. (Original) The method of claim 18 wherein the inducible promoter is a sophorose-inducible promoter.

22. (Original) The method of claim 18 wherein the inducible promoter is a gentiobiose-inducible promoter.

23. (Original) The method of claim 17 wherein the protein of interest is a heterologous protein.

24. (Currently amended) The method of claim 23 wherein the heterologous protein is selected from the group consisting of a hormone, an enzyme, a growth factor, a cytokine and an antibody ~~hormones, enzymes, growth factors, cytokines, and antibodies.~~

25. (Currently amended) The method of claim 15 wherein the cell is a filamentous fungal cell ~~fungus~~.

26. (Currently amended) The method of claim 25 wherein the filamentous fungus is selected from the group consisting of *Trichoderma*, *Humicola*, *Fusarium*, *Aspergillus*, *Neurospora*, *Penicillium*, *Cephalosporium*, *Achlya*, *Podospora*, *Endothia*, *Mucor*, *Cochliobolus* and *Pyricularia*.

27. (Currently amended) The method of claim 26 wherein the filamentous fungus is *Trichoderma spp.*

28. (Currently amended) The method of claim 27 wherein the filamentous fungus is *Trichoderma reesei*.

29. (Currently amended) The method of claim 26 wherein the filamentous fungus is *Penicillium spp.*

30. (Currently amended) The method of claim 29 wherein the filamentous fungus is *Penicillium funiculosum*.

31. (Currently amended) The method of claim 15 wherein the cell is a ~~bacteria~~ bacterial cell.

32. (Previously presented) The method of claim 31 wherein the bacteria is selected from the group consisting of *Streptomyces*, *Thermomonospora*, *Bacillus*, and *Cellulomonas*.

33. (Cancelled)

34. (Cancelled)

35. (Cancelled)

36. (Currently amended) The method of claim 15 wherein the ~~cellulase preparation~~ is provided at a total protein concentration in said first mixture ranges from about 0.5g/L to about 50g/L total protein.

37. (Currently amended) The method of claim 15 wherein the glucose first mixture is incubated ~~with the cellulase composition~~ at about 50 °C to about 70 °C.

38. (Currently amended) The method of claim 37 where in the glucose first mixture is incubated ~~with the cellulase composition~~ for between 8 hours and 7 days.

39. (Cancelled)

40. (Cancelled)

41. (Currently amended) A method for producing a protein of interest from a cell culture comprising the steps of:

(a) incubating a solution comprising from about 50% to about 70% glucose and a *Trichoderma* cellulase preparation selected from the group consisting of a whole cellulase composition or beta-glucosidase enriched cellulase composition, wherein the beta-glucosidase

activity in said solution is from 1.5 IU/ml to 180 IU/ml, at a temperature of for about 50 °C to about 70 °C for a period of about 8 hours to about 500 hours; and

(b) contacting said cell culture, wherein the cell culture comprises cells containing a nucleotide sequence encoding a protein of interest operatively linked to sophorose-inducible or gentiobiose-inducible promoter, with said solution; inducing feed in an amount effective to induce expression of a sophorose-inducible or gentiobiose-inducible protein, wherein said inducing feed has not been subjected to a purification step, thereby producing said protein of interest wherein said protein is produced.

42. (Previously presented) The method of claim 41 wherein the protein produced is an endogenous protein.

43. (Previously presented) The method of claim 41 wherein the protein produced is an endogenous cellulase.

44. (Previously presented) The method of claim 41 wherein the protein produced is a heterologous protein.

45. (Currently amended) The method of claim 44 wherein the heterologous protein is selected from the group consisting of a hormone, an enzyme, a growth factor, a cytokine and an antibody hormones, enzymes, growth factors, cytokines, and antibodies.

46. (Previously presented) The method of claim 45 wherein said enzyme is a cellulase.

47. (Currently amended) The method of claim 41 wherein said cell is a filamentous ~~fungus~~ fungal cell.

48. (Currently amended) The method of claim 47 wherein the filamentous fungus is selected from the group consisting of *Trichoderma*, *Humicola*, *Fusarium*, *Aspergillus*, *Neurospora*, *Penicillium*, *Cephalosporium*, *Achlya*, *Podospora*, *Endothia*, *Mucor*, *Cochliobolus* and *Pyricularia*.

49. (Currently amended) The method of claim 44 ~~47~~ wherein said filamentous fungus cell is *Trichoderma* spp.

50. (Currently amended) The method of claim 44 ~~47~~ wherein said filamentous fungus cell is *Trichoderma reesei*.

51. (Currently amended) The method of claim 44 ~~47~~ wherein said filamentous fungus cell is *Penicillium spp.*

52. (Currently amended) The method of claim 44 ~~47~~ wherein said filamentous fungus cell is *Penicillium funiculosum*.

53. (Currently amended) The method of claim 41 wherein the cell is a ~~bacteria~~ bacterial cell.

54. (Previously presented) The method of claim 53 wherein the bacteria is selected from the group consisting of *Streptomyces*, *Thermomonospora*, *Bacillus*, and *Cellulomonas*.

55. (New) The method of claim 36 wherein the total protein concentration in said first mixture ranges from about 2 g/L to about 10 g/L.

56. (New) The method of claim 41 wherein the total protein concentration in said solution ranges from about 0.5g/L to about 50 g/L.

57. (New) The method of claim 56 wherein the total protein concentration in said solution ranges from about 2g/L to about 10 g/L.

58. (New) The method of claim 15 wherein said inducing feed is added to said cell culture in fed batch mode.

59. (New) The method of claim 58 wherein said cell culture is cultured under conditions of carbon limitation.

60. (New) The method of claim 41 wherein said inducing feed is added to said cell culture in fed batch mode.

61. (New) The method of claim 60 wherein said cell culture is cultured under conditions of carbon limitation.

62. (New) The method of claim 15 wherein the cellulase preparation is a *Trichoderma reesei* cellulase preparation.

63. (New) The method of claim 15 in which the cellulase preparation is immobilized.
64. (New) The method of claim 41 in which the cellulase preparation is immobilized.
65. (New) The method of claim 15 wherein the first mixture is incubated at a temperature of about 50°C to about 65°C for a period of two to three days.
66. (New) The method of claim 15 wherein the first mixture is incubated at a temperature of about 65°C for a period of two to three days.
67. (New) The method of claim 41 wherein said solution is incubated at a temperature of about 50°C to about 65°C for a period of two to three days.
68. (New) The method of claim 41 wherein said solution is incubated at a temperature of about 65°C for a period of two to three days.
69. (New) The method of claim 15 in which said cellulase preparation is the product of *Trichoderma reesei* that has been engineered to overexpress beta-glucosidase relative to native levels.
70. (New) The method of claim 15, wherein said *Trichoderma reesei* has one or more endoglucanase and/or cellobiohydrolase genes deleted.
71. (New) The method of claim 41 in which said *Trichoderma reesei* cellulase preparation is the product of *Trichoderma reesei* that has been engineered to overexpress beta-glucosidase relative to native levels.
72. (New) The method of claim 41, wherein said *Trichoderma reesei* has one or more endoglucanase and/or cellobiohydrolase genes deleted.
73. (New) The method of claim 41, wherein an inducing feed composition comprising sophorose in a concentration ranging from 2 g/L to 25 g/L, gentiobiose in a concentration ranging from 35 g/L to 60 g/L, and glucose is produced in step (a).
74. (New) The method of claim 15, wherein said first mixture comprises from about 50% to about 70% glucose.

75. (New) The method of claim 15, wherein said protein of interest has an activity value of at least 1000% to 3000% greater than the activity value of a protein of interest produced by a control culture fed with glucose.

76. (New) The method of claim 41, wherein said protein of interest has an activity value of at least 1000% to 3000% greater than the activity value of a protein of interest produced by a control culture fed with glucose.

77. (New) The method of claim 41 wherein the cell has been has been genetically engineered to encode a protein of interest under the control of a sophorose-inducible promoter or a gentiobiose-inducible promoter.

78. (New) The method of claim 41 wherein the cellulase preparation is a *Trichoderma reesei* cellulase preparation.

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